

This is a repository copy of *Voltage-gated sodium channels and metastatic disease*.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/83467/>

Version: Accepted Version

---

**Article:**

Brackenbury, William J orcid.org/0000-0001-6882-3351 (2012) Voltage-gated sodium channels and metastatic disease. *Channels* (Austin). pp. 352-361. ISSN 1933-6969

<https://doi.org/10.4161/chan.21910>

---

**Reuse**

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

**Takedown**

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing [eprints@whiterose.ac.uk](mailto:eprints@whiterose.ac.uk) including the URL of the record and the reason for the withdrawal request.

## **Voltage-gated sodium channels and metastatic disease**

William J. Brackenbury\*

Department of Biology, University of York, UK

\*Correspondence to:

William J. Brackenbury, Department of Biology, University of York, Heslington,  
York, YO10 5DD, UK

Tel: +44 1904 328284

Email: [william.brackenbury@york.ac.uk](mailto:william.brackenbury@york.ac.uk)

### **Keywords**

Anticonvulsant / Cancer / Invasion / Metastasis / Migration / Phenytoin / Voltage-gated Na<sup>+</sup> channel

### **Abbreviations**

CAM, cell adhesion molecule; DHT, dihydrotestosterone; DMBA, 7,12-dimethylbenz(a) anthracene; EGF, epidermal growth factor; ER, estrogen receptor  $\alpha$ ; ERK, extracellular signal-regulated kinase; FGF1B, fibroblast growth factor-homologous factor 1B; HER2, human epidermal growth factor 2; Ig, immunoglobulin; NF, neurofascin; NGF, nerve growth factor; PKA, protein kinase A; PKC, protein kinase C; PR, progesterone receptor; RPTP $\beta$ , receptor protein tyrosine phosphatase  $\beta$ ; TTX, tetrodotoxin; VEGF, vascular endothelial growth factor; VGSC, voltage-gated Na<sup>+</sup> channel.

## Abstract

Voltage-gated Na<sup>+</sup> channels (VGSCs) are macromolecular protein complexes containing a pore-forming  $\alpha$  subunit and smaller non-pore-forming  $\beta$  subunits. VGSCs are expressed in metastatic cells from a number of cancers. In these cells, Na<sup>+</sup> current carried by  $\alpha$  subunits enhances migration, invasion and metastasis *in vivo*. In contrast, the  $\beta$  subunits mediate cellular adhesion and process extension. The prevailing hypothesis is that VGSCs are up-regulated in cancer, in general favoring an invasive/metastatic phenotype, although the mechanisms are still not fully clear. Expression of the Na<sub>v</sub>1.5  $\alpha$  subunit associates with poor prognosis in clinical breast cancer specimens, suggesting that VGSCs may have utility as prognostic markers for cancer progression. Furthermore, repurposing existing VGSC-blocking therapeutic drugs may provide a new strategy to improve outcomes in patients suffering from metastatic disease, which is the major cause of cancer-related deaths, and for which there is currently no cure.

## Introduction

Voltage-gated Na<sup>+</sup> channels (VGSCs) are comprised of a pore-forming  $\alpha$  subunit typically in association with one or more smaller  $\beta$  subunits (Figure 1).<sup>1</sup> The  $\beta$  subunits regulate channel expression and gating, and are immunoglobulin (Ig) superfamily cell adhesion molecules (CAMs).<sup>2</sup> VGSCs are classically responsible for action potential initiation and conduction in excitable cells.<sup>3</sup> Both the  $\alpha$  and  $\beta$  subunits have been shown to interact with a range of other signaling molecules (Figure 1), enabling fine-tuning of channel activity on the one hand, and allowing

VGSCs to participate in non-conducting signaling on the other.<sup>4</sup> VGSCs are also expressed in a range of cell types that are considered “non-excitables”, including glia, fibroblasts, immune cells, and metastatic cancer cells.<sup>5</sup> The past 15 years have seen a rapid expansion in published studies documenting the expression VGSCs across a broadening number of cancers, their role in regulating cellular migration and invasion, and, importantly, their potential utility as diagnostic and therapeutic targets. In particular, several recent studies have started to define a mechanistic role for VGSCs in regulating migration and invasion. The purpose of this review is to assimilate the current body of evidence ascribing a malignant role for VGSCs during metastasis (the spread of tumor cells from primary to distant sites), and consider the clinical implications.

### **VGSC expression in cancer**

The VGSC  $\alpha$  subunit family contains nine members, Nav1.1-Nav1.9, encoded by genes *SCN1A-SCN11A* (Table 1A).<sup>6</sup> There are four  $\beta$  subunits,  $\beta 1$ - $\beta 4$ , encoded by genes *SCN1B-SCN4B*.<sup>2</sup> VGSC expression has been predominantly reported to date in carcinomas (cancers of epithelial origin). The  $\alpha$  subunits have been identified in cells from the following carcinomas: breast cancer,<sup>7, 8</sup> cervical cancer,<sup>9, 10</sup> colon cancer,<sup>11</sup> melanoma<sup>12, 13</sup> mesothelioma,<sup>14</sup> neuroblastoma,<sup>15</sup> non-small cell lung cancer,<sup>16</sup> ovarian cancer,<sup>17</sup> prostate cancer,<sup>18-21</sup> and small-cell lung cancer<sup>22, 23</sup> (Table 1A).  $\alpha$  subunits are also expressed in gliomas,<sup>24, 25</sup> lymphoma<sup>26</sup> and leukemia cells,<sup>27</sup> the latter suggesting that VGSCs may be present in hematological malignancies, in addition to solid tumors. Although the majority of reports published to date have

focused on cell lines, a number of studies now show that  $\alpha$  subunits are also expressed *in vivo*, in patient biopsy material.<sup>8-11, 17, 28-30</sup>

In excitable cells/tissues, different  $\alpha$  subunits have subtly individual, but often largely overlapping tissue distributions, which are proposed to permit functional specializations as a result of subtle variations in electrophysiological properties.<sup>31</sup> In cancer cells, there appears to be a variable pattern of expression of different  $\alpha$  subunits, such that a number of cancers express multiple  $\alpha$  subunits, but not all subtypes are expressed in all cancers (Table 1A). In several cancers expressing multiple  $\alpha$  subunits, a predominant  $\alpha$  subunit has been identified. For example, in lymphoma and breast cancer cells the most highly expressed  $\alpha$  subunit is Na<sub>v</sub>1.5 (gene: *SCN5A*),<sup>8, 26</sup> whereas in prostate cancer cells the predominant  $\alpha$  subunit is Na<sub>v</sub>1.7 (gene: *SCN9A*).<sup>18</sup>

Alternative mRNA splicing enables further functional variation among  $\alpha$  subunits.<sup>32</sup> An important developmentally regulated splicing event occurs in exon 6, encoding the domain I segment 3 (DI:S3) region, such that the 5' "neonatal" variant is expressed at birth, whereas the 3' "adult" variant is expressed later in postnatal development.<sup>33</sup> In lymphoma, neuroblastoma, breast and prostate cancer cells, *SCN5A* and *SCN9A* are mainly expressed in their DI:S3 5' neonatal splice forms.<sup>8, 15, 26, 28</sup> In contrast, the adult *SCN5A* variant is expressed in colon cancer cells, and the neonatal variant is absent.<sup>11</sup> In certain cancers, the presence of  $\alpha$  subunits may therefore be an example of oncofetal gene expression, where embryonic genes are pathologically re-expressed during oncogenesis.<sup>34</sup>

In several cancers,  $\alpha$  subunit mRNA and protein expression correlates with metastatic potential. For example, in breast cancer, the neonatal *SCN5A* splice variant is expressed ~1800-fold higher in metastatic MDA-MB-231 cells than weakly metastatic MCF-7 cells.  $\text{Na}^+$  currents are detectable in MDA-MB-231 cells, but not in weakly metastatic MCF-7 cells.<sup>7, 8</sup> The expression of neonatal *SCN5A* mRNA in breast cancer biopsies correlates with occurrence of lymph node metastasis.<sup>8</sup> Furthermore, *SCN5A* mRNA is elevated in breast tumors from patients who had a recurrence, or died within five years, and associates with increased odds of developing metastasis.<sup>30</sup> A similar pattern has been observed in prostate cancer cells, where VGSC expression increases in line with metastatic potential in the LNCaP progression model.<sup>35</sup> In agreement with this, the predominant  $\alpha$  subunit, *SCN9A*, is elevated in prostate cancer biopsies compared to non-cancerous prostate samples,<sup>28</sup> and is more highly expressed in strongly metastatic PC-3 and Mat-LyLu cells than weakly metastatic LNCaP and AT-2 cells.<sup>18</sup>  $\text{Na}^+$  currents have been detected only in the metastatic prostate cancer cell lines.<sup>19, 21</sup> The positive correlation between  $\alpha$  subunit expression and metastatic potential has also been reported for colon<sup>11</sup> and ovarian cancers.<sup>17</sup> However, in gliomas, the mRNA level of  $\alpha$  subunits is inversely correlated with malignancy grade.<sup>25</sup> There appears to be no association between  $\alpha$  subunit mRNA expression and metastatic potential of small cell or non-small cell lung cancer cell lines.<sup>16, 22</sup> Therefore, the relationship between  $\alpha$  subunit expression, tumor grade and metastatic potential may be cancer type-specific.

The expression of  $\beta$  subunits in cancer cells has been less extensively studied.  $\beta$  subunits are expressed in prostate,<sup>36, 37</sup> breast,<sup>38</sup> non-small cell lung,<sup>16</sup> and cervical cancers<sup>10</sup> (Table 1B). As with the  $\alpha$  subunits, the  $\beta$  subunit expression profile appears

to vary between cancers. For example,  $\beta 3$  is present in prostate and non-small cell lung cancer cells,<sup>16, 36</sup> but is absent in breast and cervical cancer cells.<sup>10, 38</sup> However,  $\beta 1$  is the most abundant  $\beta$  subunit in breast, prostate, and cervical cancer cells.<sup>10, 36, 38</sup> Interestingly,  $\beta 1$  appears to be inversely correlated with *SCN5A* and metastatic potential in breast cancer cells: *SCN1B* mRNA (encoding  $\beta 1$ ) is significantly higher in weakly metastatic MCF-7 cells than in metastatic MDA-MB-231 cells.<sup>38</sup> This suggests that  $\beta$  subunits may be performing certain functions in cancer cells independent of the pore-forming  $\alpha$  subunits. In contrast, a recent study has shown that  $\beta 2$  expression increases in line with metastatic potential in the LNCaP prostate cancer progression model.<sup>37</sup> Therefore, as with  $\alpha$  subunits, different  $\beta$  subunits may be expressed at varying levels in different cancer types, and may perform distinct functions.

## Functional role

VGSC  $\alpha$  subunits potentiate a number of cellular behaviors associated with metastasis (Table 2). In breast, prostate and lung cancer cell lines, the VGSC pore blocker tetrodotoxin (TTX) inhibits behaviors including process outgrowth/extension,<sup>39</sup> galvanotaxis,<sup>8, 40</sup> migration,<sup>8, 14, 17, 41-44</sup> endocytosis,<sup>8, 22, 45</sup> vesicular patterning,<sup>46, 47</sup> detachment from substrate,<sup>48</sup> gene expression,<sup>43, 49</sup> and invasion.<sup>7, 8, 10-12, 16, 17, 19, 21, 26</sup> TTX does not inhibit proliferation of cancer cells,<sup>7, 8, 10, 16, 17, 41</sup> suggesting that VGSCs may be involved mainly in metastatic progression, rather than tumorigenesis.<sup>50</sup> However, recent evidence has shown that VGSCs also regulate angiogenic properties of endothelial cells, including vascular endothelial growth factor (VEGF)-induced proliferation, tubular differentiation, and adhesion.<sup>51</sup> Therefore, the exact functional

contribution of VGSCs to the cancer process may depend on the cell type, fate and state of the tumor.

Silencing *SCN5A* with siRNA reduces *in vitro* invasion of MDA-MB-231 breast cancer cells.<sup>52</sup> Further, specifically targeting the neonatal splice variant of *SCN5A* reduces the migration and invasion of MDA-MB-231 cells, suggesting that the neonatal form itself may be responsible for VGSC-dependent potentiation of metastatic cell behavior in breast cancer cells.<sup>42</sup> Similarly, siRNA targeting *SCN8A* (encoding Na<sub>v</sub>1.6) and *SCN9A* reduces invasion and endocytic activity in PC-3 metastatic prostate cancer cells.<sup>53</sup> Finally, the Na<sub>v</sub>1.6-specific toxin Cn2 inhibits the invasion of cervical cancer cells.<sup>10</sup> Thus, in different cancers, different  $\alpha$  subunits appear to promote metastatic cell behaviors. However, it is not yet clear whether expression of a specific  $\alpha$  subunit in a particular cancer provides a specific functional advantage, or is related to the natural history of the disease. The fact that any  $\alpha$  subunit is present in a cancer cell may be more important than which  $\alpha$  subunit it is. For example, overexpression of Na<sub>v</sub>1.4 in weakly metastatic LNCaP prostate cancer cells is necessary and sufficient to increase their invasiveness, even though the predominant  $\alpha$  subunit expressed in metastatic prostate cancer cells is Na<sub>v</sub>1.7.<sup>18, 35</sup>

Several studies have indicated that a number of therapeutically relevant small molecule VGSC blockers can also inhibit cell behaviors associated with metastasis. For example, the anticonvulsants phenytoin and carbamazepine inhibit secretion of prostate-specific antigen and interleukin-6 by prostate cancer cells.<sup>54</sup> Phenytoin and the local anesthetic lidocaine also inhibit endocytic activity in small cell lung cancer cells.<sup>22</sup> In addition, phenytoin suppresses migration of prostate cancer cells.<sup>41</sup>



Furthermore, we have recently shown that therapeutically relevant concentrations of phenytoin inhibit  $\text{Na}^+$  current, migration and invasion in metastatic breast cancer cells.<sup>30</sup>

### **Persistent $\text{Na}^+$ current**

Cancer cells typically have a relatively depolarized membrane potential compared to terminally differentiated cells, e.g. epithelia, neurons.<sup>55</sup> For example, the resting membrane potential of a typical neuron may be around -65 mV,<sup>56</sup> whereas we and others have shown that metastatic MDA-MB-231 breast cancer cells have membrane potentials between  $\sim -15$  to  $-30$  mV.<sup>7, 8, 30</sup> Following depolarization, VGSCs open and rapidly inactivate within a few milliseconds, remaining inactivated until the membrane repolarizes.<sup>3</sup> Therefore, in cancer cells with depolarized resting potentials, the majority of VGSCs will be inactivated. However, several VGSCs, including  $\text{Na}_v1.5$ , do not inactivate completely, and a steady-state  $\text{Na}^+$  current persists, which is typically a few percent of the peak transient current.<sup>57, 58</sup> We recently proposed that the persistent  $\text{Na}^+$  current is likely to be predominant in cancer cells expressing VGSCs, and this component of the  $\text{Na}^+$  current may specifically potentiate the cells' migration and invasion.<sup>30</sup>

### **Mechanisms of action**

The obvious question is: how does  $\text{Na}^+$  influx through VGSCs potentiate metastatic behaviors, including invasion? Over the years, there has been much speculation in the

literature (reviewed in <sup>50</sup>). Three models are considered below, based on recently published experimental data.

1. Regulation of pH. In MDA-MB-231 cells, Na<sup>+</sup> influx through Na<sub>v</sub>1.5 results in intracellular alkalization, and extracellular acidification adjacent to the plasma membrane.<sup>52</sup> The Na<sup>+</sup>/H<sup>+</sup> exchanger NHE1, which is an important regulator of H<sup>+</sup> efflux, is co-expressed with Na<sub>v</sub>1.5 in lipid rafts contained within the caveolae of MDA-MB-231 cells.<sup>59</sup> Na<sup>+</sup> influx through Na<sub>v</sub>1.5 increases H<sup>+</sup> efflux through NHE1, thus enhancing pH-dependent extracellular matrix degradation and invasion (Figure 2).<sup>59</sup> However, the precise mechanism by which Na<sub>v</sub>1.5 activity enhances NHE1 is not yet clear. The resultant (Na<sub>v</sub>1.5/NHE1-dependent) perimembrane acidification is proposed to favor the proteolytic activity of cysteine cathepsins B and S, the function of which has been shown to depend, at least in part, on VGSC function.<sup>52</sup> A similar mechanism has been identified by which Na<sub>v</sub>1.5 expressed on intracellular endosomal membranes of primed macrophages acts as a charge sink, permitting Na<sup>+</sup> efflux from the endosome, resulting in H<sup>+</sup> influx, likely via the vesicular ATPase, and subsequent endosomal acidification.<sup>60</sup>

2. Regulation of gene expression. Several studies have shown that VGSCs regulate gene expression, both in excitable cells, e.g. neurons and cardiomyocytes, and in cancer cells.<sup>38, 43, 49, 61, 62</sup> *In silico* factor graph nested effects modeling of gene expression in colon cancer cell lines has revealed a novel network of gene interactions that are implicated in cancer invasion.<sup>11</sup> Strikingly, *SCN5A* is a key regulator of this invasion gene network, suggesting that VGSCs, in particular Na<sub>v</sub>1.5, may function as early entry points in signaling mechanisms regulating invasion. Downstream gene

ontology categories include Wnt signaling, cell migration, ectoderm development, response to biotic stimulus, steroid metabolic process, and cell cycle regulation (Figure 3).<sup>11</sup> These data suggest that, at least in colon cancer, Na<sub>v</sub>1.5 may regulate invasion by mechanism(s) in addition to/instead of H<sup>+</sup> efflux.<sup>59</sup> The challenge now is to understand how Na<sup>+</sup> current mediated by Na<sub>v</sub>1.5 may regulate transcription in cancer cells, or, indeed, whether the effect is mediated by mechanism(s) independent of ion conduction.

3. Regulation of intracellular Ca<sup>2+</sup>. In excitable cells, Na<sup>+</sup> current carried by VGSCs can result in an increase in intracellular Ca<sup>2+</sup> level, e.g. by activating voltage-gated Ca<sup>2+</sup> channels.<sup>63, 64</sup> Similar VGSC-dependent elevation of intracellular Ca<sup>2+</sup> has been reported in non-excitabile cells. For example, Na<sup>+</sup> current carried by Na<sub>v</sub>1.5 is essential for the sustained Ca<sup>2+</sup> entry into CD4<sup>+</sup> T cells that occurs during positive selection.<sup>65</sup> In addition to being expressed at the plasma membrane,  $\alpha$  subunits are also present on internal membranes of cancer cells and macrophages.<sup>12, 30, 60</sup> In THP-1 macrophages and HTB-66 melanoma cells, Na<sub>v</sub>1.6 is expressed on vesicular structures adjacent to podosomes.<sup>12</sup> Agonist-mediated activation of VGSCs in these cells causes Na<sup>+</sup> release from cationic intracellular stores, followed by rapid Na<sup>+</sup> uptake by anionic mitochondria, and subsequent Ca<sup>2+</sup> release into the cytosol.<sup>12</sup> It is proposed that this Ca<sup>2+</sup> release then enhances podosome/invadopodia formation, leading to increased invasion.<sup>12</sup> However, it is not yet clear how VGSCs present on vesicular membranes are gated, and/or whether they interact with VGSCs present at the plasma membrane. In vascular endothelial cells, VGSC-mediated Na<sup>+</sup> influx is required for VEGF-induced membrane depolarization and elevation of intracellular Ca<sup>2+</sup>, which in turn activates PKC and extracellular signal-regulated kinase (ERK)1/2,

potentiating angiogenic functions including proliferation, differentiation and adhesion.<sup>51</sup> In contrast to cancer cells and macrophages, the  $\text{Ca}^{2+}$  rise in endothelial cells occurs as a result of  $\text{Ca}^{2+}$  influx through reverse mode operation of the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger NCX.<sup>66</sup>

In summary, several models have recently emerged, supported by experimental data, suggesting that VGSC  $\alpha$  subunits may enhance cellular invasion by a variety of mechanisms. Further studies will no doubt contribute further data in order to clarify whether the mechanisms discussed above are widely applicable to all cancers in which VGSCs are expressed, or whether different VGSCs perform distinct functions in different cells in different cancers.

### **The role of $\beta$ subunits**

In breast cancer,  $\beta 1$  is highly expressed in weakly metastatic MCF-7 cells, where it enhances adhesion and retards transwell migration.<sup>38</sup>  $\beta 1$  is expressed at a much lower level in strongly metastatic MDA-MB-231 cells, and stable overexpression of  $\beta 1$  in this line increases cell-cell adhesion, induces process outgrowth, and reduces migration in wound healing assays.<sup>38</sup>  $\beta 2$  appears to play a slightly different role in prostate cancer cells. Overexpression of  $\beta 2$  in LNCaP cells increases adhesion, process outgrowth, migration and invasion.<sup>37</sup> However, the same study reported that  $\beta 2$ -over-expressing LNCaP cells have a reduced tumor take and smaller tumor volume when subcutaneously injected into nude mice. Thus, not only do  $\beta 1$  and  $\beta 2$  play subtly different functional roles in different cancer cells, these subunits may function differently in tumors *in vivo*, compared to cells in culture. This highlights the

critical importance of studying the functional role of VGSCs in cancer *in vivo*, in addition to using *in vitro* models. Nonetheless, given that  $\beta$  subunits are Ig family cell adhesion molecules, it is plausible to speculate that their major contribution to the metastatic behaviors of cancer cells may be through regulated adhesion/detachment. Of special note is that  $\beta$  subunits play a critical role in regulating adhesion and migration in excitable cells, where they are normally expressed.<sup>2</sup> For example,  $\beta 1$  enhances neurite outgrowth, neuronal pathfinding and fasciculation during early postnatal central nervous system development.<sup>67, 68</sup>  $\beta 1$ -mediated neurite outgrowth in cerebellar granule neurons requires fyn kinase, the cell adhesion molecule contactin, and is dependent on  $\text{Na}^+$  current carried by  $\text{Na}_v 1.6$ .<sup>2, 68</sup> (Figure 4A,B). In turn,  $\text{Na}_v 1.6$ -dependent resurgent  $\text{Na}^+$  current and action potential firing are dependent on the presence of  $\beta 1$ .<sup>56</sup> (Figure 4B). Thus, in neurons, there is a functional relationship between  $\alpha$  and  $\beta$  subunits, such that both are required for neurite outgrowth, migration and electrical excitability. This is an interesting contrast with  $\beta 1$  and  $\text{Na}_v 1.5$  in breast cancer cells, which appear to play opposing, antagonistic roles in regulating adhesion and migration.<sup>38</sup>

$\beta$  subunits regulate the mRNA levels of  $\alpha$  subunits, including  $\text{Na}_v 1.5$ , and other  $\beta$  subunits, in neurons and cardiomyocytes.<sup>61, 69-71</sup> Down-regulation of *SCN1B* in MCF-7 cells results in an increase in the mRNA level of the neonatal *SCN5A* splice variant,<sup>38</sup> raising the possibility that  $\beta 1$  may regulate gene transcription, and supporting the notion that  $\beta 1$  and  $\text{Na}_v 1.5$  may function antagonistically in breast cancer cells.<sup>30</sup> However, their expression may not be mutually exclusive, and both subunits colocalize in lamellipodia.<sup>30</sup>

Less is known about the expression/function of the other  $\beta$  subunits ( $\beta 3$  and  $\beta 4$ ) in cancer cells. However, *SCN3B* contains two response elements to the tumor suppressor p53, and DNA damaging agents and p53 over-expression up-regulate *SCN3B* mRNA in cancer cell lines, suggesting that  $\beta 3$  may be involved in p53-dependent apoptosis.<sup>72</sup> The function of  $\beta 4$  in cancer cells has not been investigated. However, a recent study has shown that, together with  $\text{Na}_v1.5$ ,  $\beta 4$  is required for  $\text{CD4}^+$  T cell development,<sup>65</sup> suggesting that  $\beta 4$  may play an as yet unexplored role(s) in non-excitabile cells.

In summary, VGSC  $\beta$  subunits appear to play a role in regulating a number of cell behaviors associated with metastasis, including adhesion, migration and gene expression. Importantly, some of these functions oppose the effects of  $\alpha$  subunits<sup>38</sup> (Table 3). Further work is required to delineate the respective functions of  $\alpha$  and  $\beta$  subunits in cancer cells. A major challenge will be to establish whether or not  $\beta$  subunits are functioning (e.g. to regulate adhesion and migration) independently of  $\alpha$  subunits.

### **Regulation of expression**

How are VGSCs up-regulated in cancer cells? VGSCs are macromolecular signaling complexes and their activity can be regulated at multiple levels by a vast array of different interacting partners and mechanisms, which have been studied extensively in excitable cells, e.g. neurons and cardiomyocytes (Figure 1; reviewed in <sup>2, 73</sup>).

However, the mechanisms by which expression of VGSC  $\alpha$  and  $\beta$  subunits are regulated in cancer cells are not yet well understood. Serum has been shown to

modulate expression and activity of  $\alpha$  subunits in Mat-LyLu prostate cancer cells.<sup>74</sup> Subsequent studies have shown that several components of serum have various effects on VGSC function. For example, epidermal growth factor (EGF) increases  $\text{Na}_v1.7$  expression,  $\text{Na}^+$  current and VGSC-dependent migration and invasion of Mat-LyLu and PC-3M prostate cancer cells.<sup>44, 75</sup> In addition, nerve growth factor (NGF) increases  $\text{Na}^+$  current in Mat-LyLu cells via protein kinase A (PKA) activation.<sup>76</sup> Although NGF also increases migration in these cells, its effect is independent of VGSC activity,<sup>76</sup> suggesting that different growth factors may regulate different pools of VGSCs, which may in turn have different downstream effects on metastatic cell behavior.

Steroid hormones have also been shown to regulate VGSC expression and activity in cancer cells. In MDA-MB-231 breast cancer cells, the estrogen  $\beta$ -estradiol increases  $\text{Na}^+$  current via the G-protein-coupled estrogen receptor GPR30 and PKA.<sup>77</sup> In addition, *SCN1B* expression is negatively regulated by the androgen dihydrotestosterone (DHT) in LNCaP and PC-3 prostate cancer cells.<sup>36</sup> However, there is no association between *SCN5A* expression and estrogen receptor  $\alpha$  (ER), progesterone receptor (PR), or human epidermal growth factor 2 (HER2) status in clinical breast cancer specimens,<sup>30</sup> suggesting that any relationship between serum factors and VGSC expression *in vivo* may be highly complex.

Finally,  $\alpha$  subunit expression and activity in metastatic cancer cells appears to be auto-regulated via a positive feedback mechanism. In Mat-LyLu cells,  $\text{Na}^+$  current activates PKA, which in turn up-regulates *SCN9A* mRNA levels, and promotes externalization of  $\alpha$  subunits to the plasma membrane.<sup>43</sup> Similarly, in MDA-MB-231

breast cancer cells, expression of the neonatal Na<sub>v</sub>1.5 splice variant is maintained by positive feedback involving PKA.<sup>78</sup> In both cases, inhibition of Na<sup>+</sup> current with TTX effectively collapses the positive feedback loop, suppressing VGSC-dependent migration.<sup>43, 78</sup> In summary, expression and function of VGSC  $\alpha$  and  $\beta$  subunits in cancer cells is regulated by serum components, including steroid hormones and growth factors.

### **Therapeutic potential**

The observation that VGSCs play a pro-invasive role in metastatic cells, together with patient data associating  $\alpha$  subunit expression with poor clinical outcome, suggests that VGSCs may be important diagnostic and/or therapeutic targets in the metastatic setting. Given that  $\alpha$  subunits are regulated by activity-dependent positive feedback, pharmacological blockade of channel conductance may be an ideal intervention to inhibit VGSC-dependent metastatic cell behaviors. Indeed, a recent study shows that local injection of TTX into subcutaneous tumors of Mat-LyLu cells in the Copenhagen rat significantly reduces lung metastases and improves overall survival.<sup>79</sup> However, the toxicity of TTX would preclude its use as a systemic anti-metastatic treatment.

We have proposed that VGSC-blocking drugs FDA-approved for treating other conditions, e.g. anticonvulsants, antiarrhythmics, local anesthetics, and tricyclic antidepressants, may warrant investigation for repurposing to metastatic disease.<sup>30</sup> In support of this, several of these agents inhibit metastatic cell behaviors *in vitro* models.<sup>8, 22, 30, 41, 54</sup> Application of local anesthetics during radical prostatectomy



surgery associates with significantly reduced recurrence and metastasis.<sup>80</sup> In addition, a recent retrospective case-control analysis using the General Practice Research Database suggests that use of tricyclic antidepressants may have potential for prevention of glioma and colorectal cancer.<sup>81</sup> Furthermore, riluzole, which is both a VGSC blocker and metabotropic glutamate receptor inhibitor, reduces breast cancer tumor volume in mice, and suppresses metabolic activity of tumors in patients with resectable stage III and IV melanoma.<sup>82, 83</sup> Finally, a mexiletine analog (RS100642) reduces oxidative stress associated with tumor burden, and increases overall survival in the experimentally induced 7,12-dimethylbenz(a) anthracene (DMBA) rat breast cancer model<sup>84</sup>. In summary, several studies suggest that VGSC  $\alpha$  subunits may be useful therapeutic targets in cancer.

## **Conclusion and perspectives**

A growing body of evidence suggests that VGSCs play an important pathological role during cancer progression towards metastasis. However, the role of individual  $\alpha$  and  $\beta$  subunits appears to be complex. Nonetheless, a common theme is that  $\text{Na}^+$  current carried by  $\alpha$  subunits favors an invasive phenotype, whereas  $\beta$  subunits may regulate adhesion. Future work is required to establish how widely VGSCs are expressed across different types of cancer, and the extent of contribution(s) of different  $\alpha$  and  $\beta$  subunits to disease progression. The ultimate goal should be to definitively evaluate the potential for both  $\alpha$  and  $\beta$  subunits as diagnostic and therapeutic targets. In respect of the former, repurposing FDA-approved channel blockers may be a cost-effective intervention in metastatic disease, which is the major cause of cancer-related deaths<sup>85</sup>,<sup>86</sup>, and treatment of which is still largely limited to palliation<sup>87</sup>.

## Acknowledgements

This work was supported by the Medical Research Council [Fellowship number G1000508(95657)].

## Conflict of interest

The author declares no conflicts of interest.

## References

1. Catterall WA. Cellular and molecular biology of voltage-gated sodium channels. *Physiological Reviews* 1992; 72:S15-S48.
2. Brackenbury WJ, Isom LL. Na Channel beta Subunits: Overachievers of the Ion Channel Family. *Front Pharmacol* 2011; 2:53.
3. Hille B. Ionic channels of excitable membranes. Sunderland (Massachusetts): Sinauer Associates Inc., 1992.
4. Brackenbury WJ, Isom LL. Voltage-gated Na<sup>+</sup> channels: potential for beta subunits as therapeutic targets. *Expert Opin Ther Targets* 2008; 12:1191-203.
5. Brackenbury WJ, Djamgoz MB, Isom LL. An emerging role for voltage-gated Na<sup>+</sup> channels in cellular migration: Regulation of central nervous system development and potentiation of invasive cancers. *The Neuroscientist* 2008; 14:571-83.
6. Catterall WA. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. *Neuron* 2000; 26:13-25.
7. Roger S, Besson P, Le Guennec JY. Involvement of a novel fast inward sodium current in the invasion capacity of a breast cancer cell line. *Biochim Biophys Acta* 2003; 1616:107-11.
8. Fraser SP, Diss JK, Chioni AM, Mycielska M, Pan H, Yamaci RF, et al. Voltage-gated sodium channel expression and potentiation of human breast cancer metastasis. *Clin Cancer Res* 2005; 11:5381-9.
9. Diaz D, Delgadillo DM, Hernandez-Gallegos E, Ramirez-Dominguez ME, Hinojosa LM, Ortiz CS, et al. Functional expression of voltage-gated sodium channels in primary cultures of human cervical cancer. *J Cell Physiol* 2007; 210:469-78.
10. Hernandez-Plata E, Ortiz CS, Marquina-Castillo B, Medina-Martinez I, Alfaro A, Berumen J, et al. Overexpression of Na(V) 1.6 channels is associated with the invasion capacity of human cervical cancer. *Int J Cancer* 2012; 130:2013-23.

11. House CD, Vaske CJ, Schwartz A, Obias V, Frank B, Luu T, et al. Voltage-gated Na<sup>+</sup> channel SCN5A is a key regulator of a gene transcriptional network that controls colon cancer invasion. *Cancer Res* 2010; 70:6957-67.
12. Carrithers MD, Chatterjee G, Carrithers LM, Offoha R, Iheagwara U, Rahner C, et al. Regulation of podosome formation in macrophages by a novel splice variant of the sodium channel SCN8A. *J Biol Chem* 2009; 284:8114-26.
13. Allen DH, Lepple-Wienhues A, Cahalan MD. Ion channel phenotype of melanoma cell lines. *J Membr Biol* 1997; 155:27-34.
14. Fulgenzi G, Graciotti L, Faronato M, Soldovieri MV, Miceli F, Amoroso S, et al. Human neoplastic mesothelial cells express voltage-gated sodium channels involved in cell motility. *The international journal of biochemistry & cell biology* 2006; 38:1146-59.
15. Ou SW, Kameyama A, Hao LY, Horiuchi M, Minobe E, Wang WY, et al. Tetrodotoxin-resistant Na<sup>+</sup> channels in human neuroblastoma cells are encoded by new variants of Nav1.5/SCN5A. *Eur J Neurosci* 2005; 22:793-801.
16. Roger S, Rollin J, Barascu A, Besson P, Raynal PI, Iochmann S, et al. Voltage-gated sodium channels potentiate the invasive capacities of human non-small-cell lung cancer cell lines. *The international journal of biochemistry & cell biology* 2007; 39:774-86.
17. Gao R, Shen Y, Cai J, Lei M, Wang Z. Expression of voltage-gated sodium channel alpha subunit in human ovarian cancer. *Oncol Rep* 2010; 23:1293-9.
18. Diss JK, Archer SN, Hirano J, Fraser SP, Djamgoz MB. Expression profiles of voltage-gated Na<sup>+</sup> channel alpha-subunit genes in rat and human prostate cancer cell lines. *The Prostate* 2001; 48:165-78.
19. Laniado ME, Lalani EN, Fraser SP, Grimes JA, Bhargal G, Djamgoz MB, et al. Expression and functional analysis of voltage-activated Na<sup>+</sup> channels in human prostate cancer cell lines and their contribution to invasion in vitro. *Am J Pathol* 1997; 150:1213-21.
20. Smith P, Rhodes NP, Shortland AP, Fraser SP, Djamgoz MB, Ke Y, et al. Sodium channel protein expression enhances the invasiveness of rat and human prostate cancer cells. *FEBS Lett* 1998; 423:19-24.
21. Grimes JA, Fraser SP, Stephens GJ, Downing JE, Laniado ME, Foster CS, et al. Differential expression of voltage-activated Na<sup>+</sup> currents in two prostatic tumour cell lines: contribution to invasiveness in vitro. *FEBS Lett* 1995; 369:290-4.
22. Onganer PU, Djamgoz MB. Small-cell lung cancer (human): potentiation of endocytic membrane activity by voltage-gated Na<sup>+</sup> channel expression in vitro. *J Membr Biol* 2005; 204:67-75.
23. Blandino JK, Viglione MP, Bradley WA, Oie HK, Kim YI. Voltage-dependent sodium channels in human small-cell lung cancer cells: role in action potentials and inhibition by Lambert-Eaton syndrome IgG. *J Membr Biol* 1995; 143:153-63.
24. Joshi AD, Parsons DW, Velculescu VE, Riggins GJ. Sodium ion channel mutations in glioblastoma patients correlate with shorter survival. *Mol Cancer* 2011; 10:17.
25. Schrey M, Codina C, Kraft R, Beetz C, Kalff R, Wolfl S, et al. Molecular characterization of voltage-gated sodium channels in human gliomas. *Neuroreport* 2002; 13:2493-8.
26. Fraser SP, Diss JK, Lloyd LJ, Pani F, Chioni AM, George AJ, et al. T-lymphocyte invasiveness: control by voltage-gated Na<sup>+</sup> channel activity. *FEBS Lett* 2004; 569:191-4.

27. Yamashita N, Hamada H, Tsuruo T, Ogata E. Enhancement of voltage-gated Na<sup>+</sup> channel current associated with multidrug resistance in human leukemia cells. *Cancer Res* 1987; 47:3736-41.
28. Diss JK, Stewart D, Pani F, Foster CS, Walker MM, Patel A, et al. A potential novel marker for human prostate cancer: voltage-gated sodium channel expression in vivo. *Prostate cancer and prostatic diseases* 2005; 8:266-73.
29. Onganer PU, Seckl MJ, Djamgoz MB. Neuronal characteristics of small-cell lung cancer. *Br J Cancer* 2005; 93:1197-201.
30. Yang M, Kozminski DJ, Wold LA, Modak R, Calhoun JD, Isom LL, et al. Therapeutic potential for phenytoin: targeting Na(v)1.5 sodium channels to reduce migration and invasion in metastatic breast cancer. *Breast Cancer Res Treat* 2012; 134:603-15.
31. Clare JJ, Tate SN, Nobbs M, Romanos MA. Voltage-gated sodium channels as therapeutic targets. *Drug Discov Today* 2000; 5:506-20.
32. Diss JK, Fraser SP, Djamgoz MB. Voltage-gated Na<sup>+</sup> channels: multiplicity of expression, plasticity, functional implications and pathophysiological aspects. *European biophysics journal : EBJ* 2004; 33:180-93.
33. Onkal R, Mattis JH, Fraser SP, Diss JK, Shao D, Okuse K, et al. Alternative splicing of Nav1.5: An electrophysiological comparison of 'neonatal' and 'adult' isoforms and critical involvement of a lysine residue. *J Cell Physiol* 2008; 216:716-26.
34. Monk M, Holding C. Human embryonic genes re-expressed in cancer cells. *Oncogene* 2001; 20:8085-91.
35. Bennett ES, Smith BA, Harper JM. Voltage-gated Na<sup>+</sup> channels confer invasive properties on human prostate cancer cells. *Pflugers Arch* 2004; 447:908-14.
36. Diss JK, Fraser SP, Walker MM, Patel A, Latchman DS, Djamgoz MB. Beta-subunits of voltage-gated sodium channels in human prostate cancer: quantitative in vitro and in vivo analyses of mRNA expression. *Prostate cancer and prostatic diseases* 2008; 11:325-33.
37. Jansson KH, Lynch JE, Lepori-Bui N, Czymmek KJ, Duncan RL, Sikes RA. Overexpression of the VSSC-associated CAM, beta-2, enhances LNCaP cell metastasis associated behavior. *The Prostate* 2012; 72:1080-92.
38. Chioni AM, Brackenbury WJ, Calhoun JD, Isom LL, Djamgoz MB. A novel adhesion molecule in human breast cancer cells: Voltage-gated Na<sup>+</sup> channel  $\beta$  1 subunit. *The international journal of biochemistry & cell biology* 2009; 41:1216-27.
39. Fraser SP, Ding Y, Liu A, Foster CS, Djamgoz MB. Tetrodotoxin suppresses morphological enhancement of the metastatic MAT-LyLu rat prostate cancer cell line. *Cell Tissue Res* 1999; 295:505-12.
40. Djamgoz MBA, Mycielska M, Madeja Z, Fraser SP, Korohoda W. Directional movement of rat prostate cancer cells in direct-current electric field: involvement of voltage gated Na<sup>+</sup> channel activity. *J Cell Sci* 2001; 114:2697-705.
41. Fraser SP, Salvador V, Manning EA, Mizal J, Altun S, Raza M, et al. Contribution of functional voltage-gated Na<sup>+</sup> channel expression to cell behaviors involved in the metastatic cascade in rat prostate cancer: I. lateral motility. *J Cell Physiol* 2003; 195:479-87.
42. Brackenbury WJ, Chioni AM, Diss JK, Djamgoz MB. The neonatal splice variant of Nav1.5 potentiates in vitro metastatic behaviour of MDA-MB-231 human breast cancer cells. *Breast Cancer Res Treat* 2007; 101:149-60.

43. Brackenbury WJ, Djamgoz MB. Activity-dependent regulation of voltage-gated Na<sup>+</sup> channel expression in Mat-LyLu rat prostate cancer cell line. *J Physiol* 2006; 573:343-56.
44. Onganer PU, Djamgoz MB. Epidermal growth factor potentiates in vitro metastatic behaviour of human prostate cancer PC-3M cells: Involvement of voltage-gated sodium channel. *Mol Cancer* 2007; 6:76.
45. Mycielska ME, Fraser SP, Szatkowski M, Djamgoz MB. Contribution of functional voltage-gated Na<sup>+</sup> channel expression to cell behaviors involved in the metastatic cascade in rat prostate cancer: II. Secretory membrane activity. *J Cell Physiol* 2003; 195:461-9.
46. Krasowska M, Grzywna ZJ, Mycielska ME, Djamgoz MB. Patterning of endocytic vesicles and its control by voltage-gated Na<sup>+</sup> channel activity in rat prostate cancer cells: fractal analyses. *European biophysics journal : EBJ* 2004; 33:535-42.
47. Krasowska M, Grzywna ZJ, Mycielska ME, Djamgoz MB. Fractal analysis and ionic dependence of endocytotic membrane activity of human breast cancer cells. *European biophysics journal : EBJ* 2009; 38:1115-25.
48. Palmer CP, Mycielska ME, Burcu H, Osman K, Collins T, Beckerman R, et al. Single cell adhesion measuring apparatus (SCAMA): application to cancer cell lines of different metastatic potential and voltage-gated Na<sup>+</sup> channel expression. *European biophysics journal : EBJ* 2008; 37:359-68.
49. Mycielska ME, Palmer CP, Brackenbury WJ, Djamgoz MB. Expression of Na<sup>+</sup>-dependent citrate transport in a strongly metastatic human prostate cancer PC-3M cell line: regulation by voltage-gated Na<sup>+</sup> channel activity. *J Physiol* 2005; 563:393-408.
50. Onkal R, Djamgoz MB. Molecular pharmacology of voltage-gated sodium channel expression in metastatic disease: clinical potential of neonatal Nav1.5 in breast cancer. *Eur J Pharmacol* 2009; 625:206-19.
51. Andrikopoulos P, Fraser SP, Patterson L, Ahmad Z, Burcu H, Ottaviani D, et al. Angiogenic Functions of Voltage-gated Na<sup>+</sup> Channels in Human Endothelial Cells: Modulation of vascular endothelial growth factor (VEGF) signaling. *J Biol Chem* 2011; 286:16846-60.
52. Gillet L, Roger S, Besson P, Lecaille F, Gore J, Bougnoux P, et al. Voltage-gated Sodium Channel Activity Promotes Cysteine Cathepsin-dependent Invasiveness and Colony Growth of Human Cancer Cells. *J Biol Chem* 2009; 284:8680-91.
53. Nakajima T, Kubota N, Tsutsumi T, Oguri A, Imuta H, Jo T, et al. Eicosapentaenoic acid inhibits voltage-gated sodium channels and invasiveness in prostate cancer cells. *British journal of pharmacology* 2009; 156:420-31.
54. Abdul M, Hoosein N. Inhibition by anticonvulsants of prostate-specific antigen and interleukin-6 secretion by human prostate cancer cells. *Anticancer Res* 2001; 21:2045-8.
55. Kunzelmann K. Ion channels and cancer. *J Membr Biol* 2005; 205:159-73.
56. Brackenbury WJ, Calhoun JD, Chen C, Miyazaki H, Nukina N, Oyama F, et al. Functional reciprocity between Na<sup>+</sup> channel Nav1.6 and  $\beta$  1 subunits in the coordinated regulation of excitability and neurite outgrowth. *Proc Natl Acad Sci U S A* 2010; 107:2283-8.
57. Crill WE. Persistent sodium current in mammalian central neurons. *Annu Rev Physiol* 1996; 58:349-62.
58. Ju YK, Saint DA, Gage PW. Hypoxia increases persistent sodium current in rat ventricular myocytes. *J Physiol* 1996; 497:337-47.

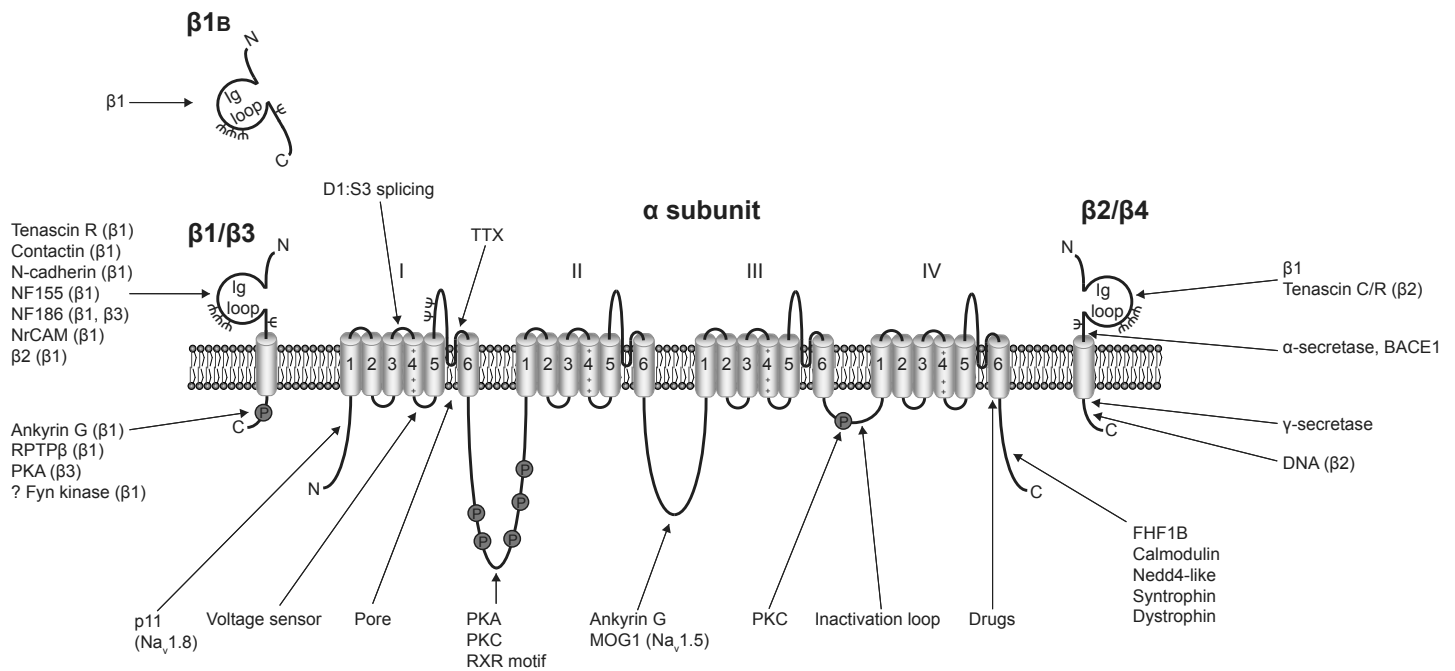
59. Brisson L, Gillet L, Calaghan S, Besson P, Le Guennec JY, Roger S, et al. Na(V)1.5 enhances breast cancer cell invasiveness by increasing NHE1-dependent H(+) efflux in caveolae. *Oncogene* 2011; 30:2070-6.
60. Carrithers MD, Dib-Hajj S, Carrithers LM, Tokmoulina G, Pypaert M, Jonas EA, et al. Expression of the voltage-gated sodium channel NaV1.5 in the macrophage late endosome regulates endosomal acidification. *J Immunol* 2007; 178:7822-32.
61. Lopez-Santiago LF, Meadows LS, Ernst SJ, Chen C, Malhotra JD, McEwen DP, et al. Sodium channel *Scn1b* null mice exhibit prolonged QT and RR intervals. *J Mol Cell Cardiol* 2007; 43:636-47.
62. Tolon RM, Sanchez-Franco F, Lopez Fernandez J, Lorenzo MJ, Vazquez GF, Cacicedo L. Regulation of somatostatin gene expression by veratridine-induced depolarization in cultured fetal cerebrocortical cells. *Brain Res Mol Brain Res* 1996; 35:103-10.
63. Dravid SM, Baden DG, Murray TF. Brevetoxin activation of voltage-gated sodium channels regulates Ca dynamics and ERK1/2 phosphorylation in murine neocortical neurons. *J Neurochem* 2004; 89:739-49.
64. Fekete A, Franklin L, Ikemoto T, Rozsa B, Lendvai B, Sylvester Vizi E, et al. Mechanism of the persistent sodium current activator veratridine-evoked Ca elevation: implication for epilepsy. *J Neurochem* 2009; 111:745-56.
65. Lo WL, Donermeyer DL, Allen PM. A voltage-gated sodium channel is essential for the positive selection of CD4(+) T cells. *Nature immunology* 2012.
66. Andrikopoulos P, Baba A, Matsuda T, Djamgoz MB, Yaqoob MM, Eccles SA. Ca<sup>2+</sup> influx through reverse mode Na<sup>+</sup>/Ca<sup>2+</sup> exchange is critical for vascular endothelial growth factor-mediated extracellular signal-regulated kinase (ERK) 1/2 activation and angiogenic functions of human endothelial cells. *J Biol Chem* 2011; 286:37919-31.
67. Davis TH, Chen C, Isom LL. Sodium Channel  $\beta$  1 Subunits Promote Neurite Outgrowth in Cerebellar Granule Neurons. *Journal of Biological Chemistry* 2004; 279:51424-32.
68. Brackenbury WJ, Davis TH, Chen C, Slat EA, Detrow MJ, Dickendesher TL, et al. Voltage-gated Na<sup>+</sup> channel  $\beta$  1 subunit-mediated neurite outgrowth requires fyn kinase and contributes to central nervous system development in vivo. *J Neurosci* 2008; 28:3246-56.
69. Lopez-Santiago LF, Pertin M, Morisod X, Chen C, Hong S, Wiley J, et al. Sodium channel  $\beta$ 2 subunits regulate tetrodotoxin-sensitive sodium channels in small dorsal root ganglion neurons and modulate the response to pain. *J Neurosci* 2006; 26:7984-94.
70. Chen C, Westenbroek RE, Xu X, Edwards CA, Sorenson DR, Chen Y, et al. Mice lacking sodium channel  $\beta$ 1 subunits display defects in neuronal excitability, sodium channel expression, and nodal architecture. *J Neurosci* 2004; 24:4030-42.
71. Kim DY, Carey BW, Wang H, Ingano LA, Binshtok AM, Wertz MH, et al. BACE1 regulates voltage-gated sodium channels and neuronal activity. *Nat Cell Biol* 2007; 9:755-64.
72. Adachi K, Toyota M, Sasaki Y, Yamashita T, Ishida S, Ohe-Toyota M, et al. Identification of SCN3B as a novel p53-inducible proapoptotic gene. *Oncogene* 2004; 23:7791-8.
73. Abriel H. Cardiac sodium channel Na(v)1.5 and interacting proteins: Physiology and pathophysiology. *J Mol Cell Cardiol* 2010; 48:2-11.

74. Ding Y, Djamgoz MB. Serum concentration modifies amplitude and kinetics of voltage-gated Na<sup>+</sup> current in the Mat-LyLu cell line of rat prostate cancer. *The international journal of biochemistry & cell biology* 2004; 36:1249-60.
75. Ding Y, Brackenbury WJ, Onganer PU, Montano X, Porter LM, Bates LF, et al. Epidermal growth factor upregulates motility of Mat-LyLu rat prostate cancer cells partially via voltage-gated Na<sup>+</sup> channel activity. *J Cell Physiol* 2008; 215:77-81.
76. Brackenbury WJ, Djamgoz MB. Nerve growth factor enhances voltage-gated Na<sup>+</sup> channel activity and Transwell migration in Mat-LyLu rat prostate cancer cell line. *J Cell Physiol* 2007; 210:602-8.
77. Fraser SP, Ozerlat-Gunduz I, Onkal R, Diss JK, Latchman DS, Djamgoz MB. Estrogen and non-genomic upregulation of voltage-gated Na(+) channel activity in MDA-MB-231 human breast cancer cells: role in adhesion. *J Cell Physiol* 2010; 224:527-39.
78. Chioni AM, Shao D, Grose R, Djamgoz MB. Protein kinase A and regulation of neonatal Nav1.5 expression in human breast cancer cells: activity-dependent positive feedback and cellular migration. *The international journal of biochemistry & cell biology* 2010; 42:346-58.
79. Yildirim S, Altun S, Gumushan H, Patel A, Djamgoz MB. Voltage-gated sodium channel activity promotes prostate cancer metastasis in vivo. *Cancer Lett* 2012; 323:58-61.
80. Biki B, Mascha E, Moriarty DC, Fitzpatrick JM, Sessler DI, Buggy DJ. Anesthetic technique for radical prostatectomy surgery affects cancer recurrence: a retrospective analysis. *Anesthesiology* 2008; 109:180-7.
81. Walker AJ, Card T, Bates TE, Muir K. Tricyclic antidepressants and the incidence of certain cancers: a study using the GPRD. *Br J Cancer* 2011; 104:193-7.
82. Yip D, Le MN, Chan JL, Lee JH, Mehnert JA, Yudd A, et al. A phase 0 trial of riluzole in patients with resectable stage III and IV melanoma. *Clin Cancer Res* 2009; 15:3896-902.
83. Speyer CL, Smith JS, Banda M, Devries JA, Mekani T, Gorski DH. Metabotropic glutamate receptor-1: a potential therapeutic target for the treatment of breast cancer. *Breast Cancer Res Treat* 2012; 132:565-73.
84. Batcioglu K, Uyumlu AB, Satilmis B, Yildirim B, Yucel N, Demirtas H, et al. Oxidative Stress in the in vivo DMBA Rat Model of Breast Cancer: Suppression by a Voltage-gated Sodium Channel Inhibitor (RS100642). *Basic & clinical pharmacology & toxicology* 2012; 111:137-41.
85. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; 61:69-90.
86. Rugo HS. The importance of distant metastases in hormone-sensitive breast cancer. *Breast* 2008; 17 Suppl 1:S3-8.
87. Suva LJ, Griffin RJ, Makhoul I. Mechanisms of bone metastases of breast cancer. *Endocr Relat Cancer* 2009; 16:703-13.
88. Isom LL, De Jongh KS, Catterall WA. Auxiliary subunits of voltage-gated ion channels. *Neuron* 1994; 12:1183-94.
89. Patino GA, Brackenbury WJ, Bao Y, Lopez-Santiago LF, O'Malley HA, Chen C, et al. Voltage-gated Na<sup>+</sup> channel  $\beta$  1B: a secreted cell adhesion molecule involved in human epilepsy. *J Neurosci* 2011; 31:14577-91.
90. Okuse K, Malik-Hall M, Baker MD, Poon WY, Kong H, Chao MV, et al. Annexin II light chain regulates sensory neuron-specific sodium channel expression. *Nature* 2002; 417:653-6.

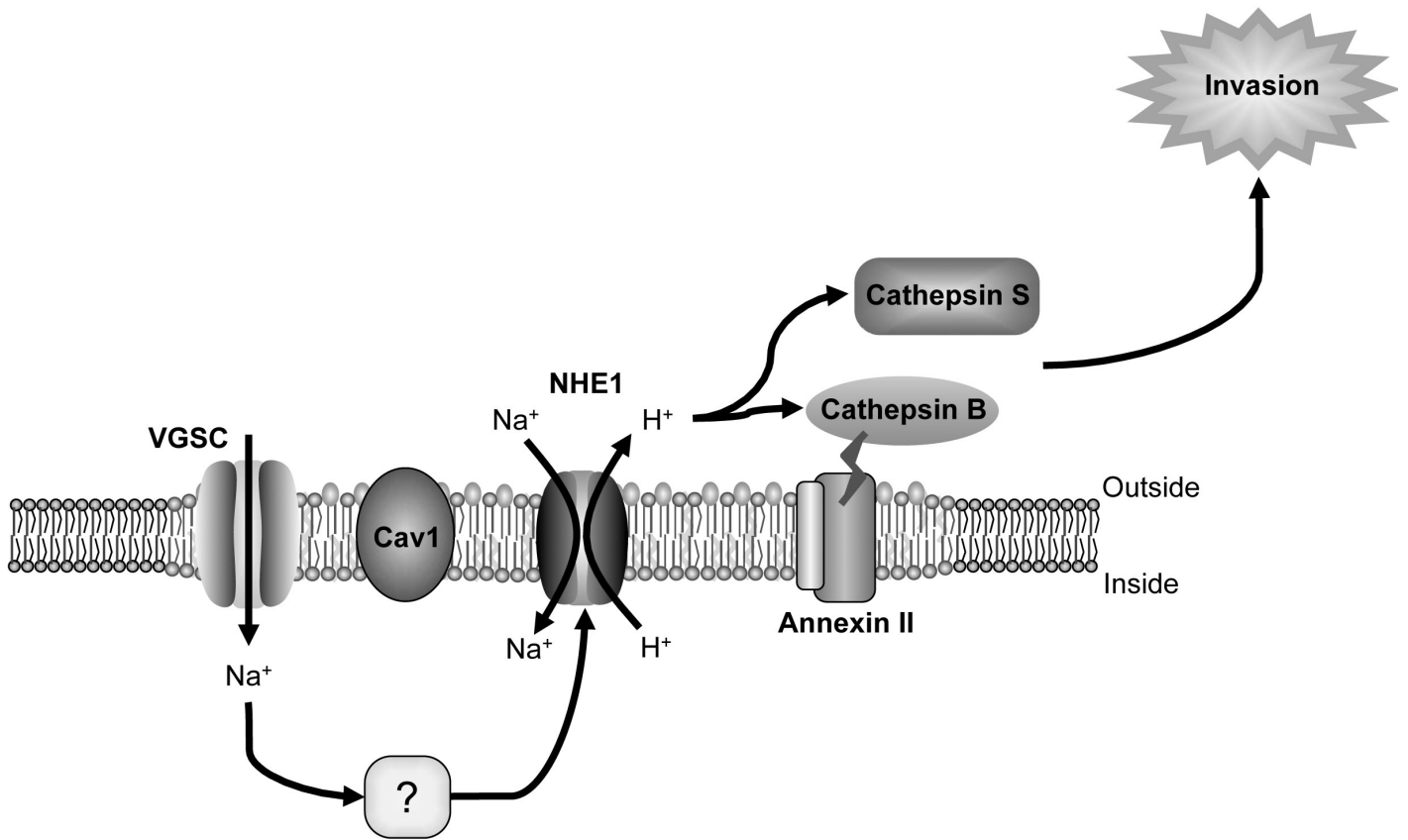
91. Cantrell AR, Smith RD, Goldin AL, Scheuer T, Catterall WA. Dopaminergic modulation of sodium current in hippocampal neurons via cAMP-dependent phosphorylation of specific sites in the sodium channel alpha subunit. *J Neurosci* 1997; 17:7330-8.
92. Numann R, Catterall WA, Scheuer T. Functional modulation of brain sodium channels by protein kinase C phosphorylation. *Science* 1991; 254:115-8.
93. Lemailet G, Walker B, Lambert S. Identification of a conserved ankyrin-binding motif in the family of sodium channel alpha subunits. *J Biol Chem* 2003; 278:27333-9.
94. Wu L, Yong SL, Fan C, Ni Y, Yoo S, Zhang T, et al. Identification of a new co-factor, MOG1, required for the full function of cardiac sodium channel Nav 1.5. *J Biol Chem* 2008; 283:6968-78.
95. Liu C, Dib-Hajj SD, Waxman SG. Fibroblast growth factor homologous factor 1B binds to the C terminus of the tetrodotoxin-resistant sodium channel rNav1.9a (NaN). *J Biol Chem* 2001; 276:18925-33.
96. Deschenes I, Neyroud N, DiSilvestre D, Marban E, Yue DT, Tomaselli GF. Isoform-specific modulation of voltage-gated Na(+) channels by calmodulin. *Circ Res* 2002; 90:E49-57.
97. van Bemmelen MX, Rougier JS, Gavillet B, Apotheloz F, Daidie D, Tateyama M, et al. Cardiac voltage-gated sodium channel Nav1.5 is regulated by Nedd4-2 mediated ubiquitination. *Circ Res* 2004; 95:284-91.
98. Abriel H, Kamynina E, Horisberger JD, Staub O. Regulation of the cardiac voltage-gated Na<sup>+</sup> channel (H1) by the ubiquitin-protein ligase Nedd4. *FEBS Lett* 2000; 466:377-80.
99. Gee SH, Madhavan R, Levinson SR, Caldwell JH, Sealock R, Froehner SC. Interaction of muscle and brain sodium channels with multiple members of the syntrophin family of dystrophin-associated proteins. *J Neurosci* 1998; 18:128-37.
100. Gavillet B, Rougier JS, Domenighetti AA, Behar R, Boixel C, Ruchat P, et al. Cardiac sodium channel Nav1.5 is regulated by a multiprotein complex composed of syntrophins and dystrophin. *Circ Res* 2006; 99:407-14.
101. Srinivasan J, Schachner M, Catterall WA. Interaction of voltage-gated sodium channels with the extracellular matrix molecules tenascin-C and tenascin-R. *Proc Natl Acad Sci U S A* 1998; 95:15753-7.
102. Xiao ZC, Ragsdale DS, Malhotra JD, Mattei LN, Braun PE, Schachner M, et al. Tenascin-R is a functional modulator of sodium channel beta subunits. *J Biol Chem* 1999; 274:26511-7.
103. Kazarinova-Noyes K, Malhotra JD, McEwen DP, Mattei LN, Berglund EO, Ranscht B, et al. Contactin associates with Na<sup>+</sup> channels and increases their functional expression. *J Neurosci* 2001; 21:7517-25.
104. Malhotra JD, Thyagarajan V, Chen C, Isom LL. Tyrosine-phosphorylated and nonphosphorylated sodium channel beta1 subunits are differentially localized in cardiac myocytes. *J Biol Chem* 2004; 279:40748-54.
105. McEwen DP, Isom LL. Heterophilic interactions of sodium channel beta1 subunits with axonal and glial cell adhesion molecules. *J Biol Chem* 2004; 279:52744-52.
106. Ratcliffe CF, Qu Y, McCormick KA, Tibbs VC, Dixon JE, Scheuer T, et al. A sodium channel signaling complex: modulation by associated receptor protein tyrosine phosphatase b. *Nature Neurosci* 2000; 3:437-44.



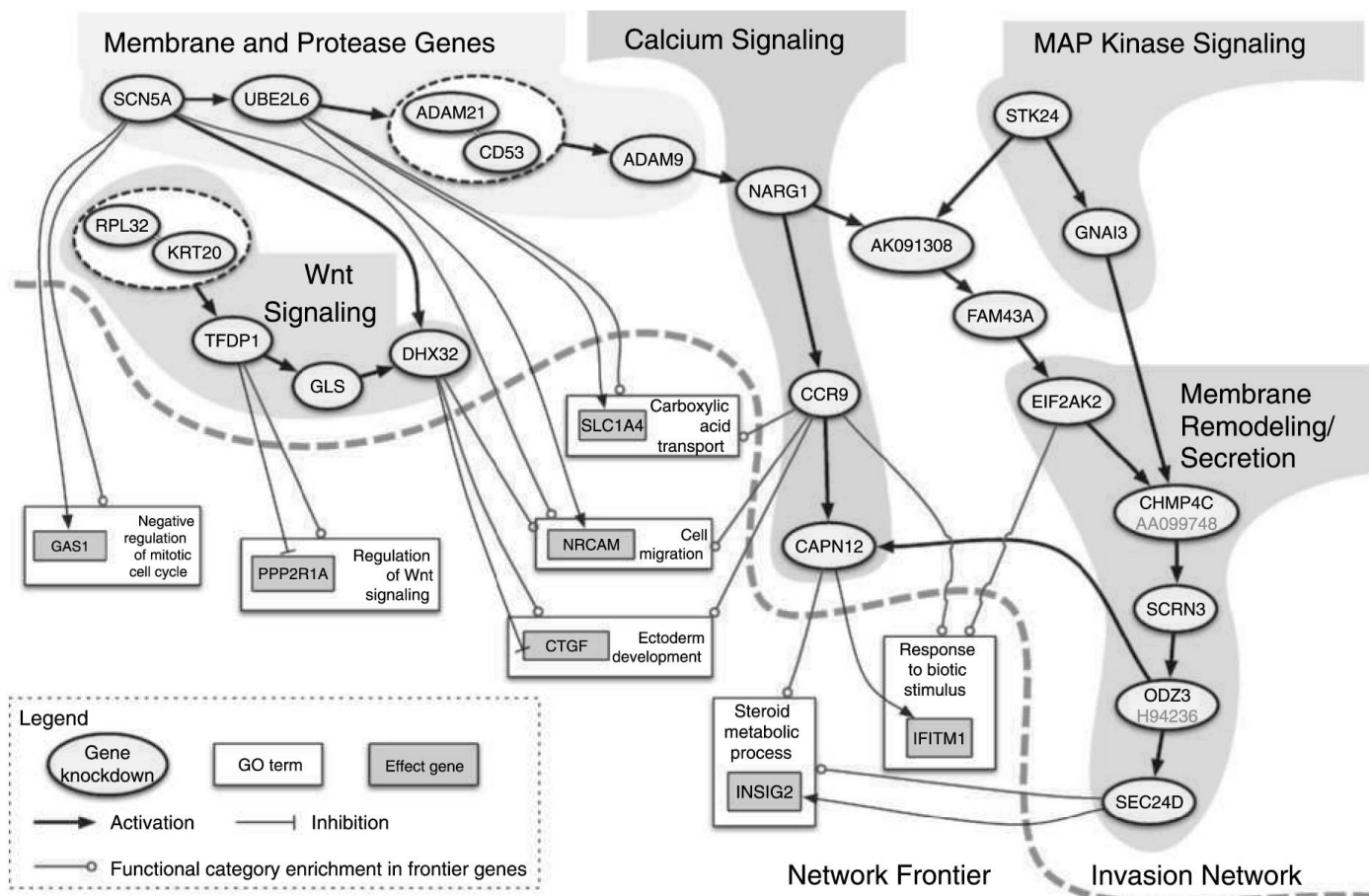
107. Merrick EC, Kalmar CL, Snyder SL, Cusdin FS, Yu EJ, Sando JJ, et al. The importance of serine 161 in the sodium channel beta3 subunit for modulation of Na(V)1.2 gating. *Pflugers Arch* 2010; 460:743-53.
108. Wong HK, Sakurai T, Oyama F, Kaneko K, Wada K, Miyazaki H, et al. beta subunits of voltage-gated sodium channels are novel substrates of BACE1 and gamma-secretase. *J Biol Chem* 2005; 280:23009-17.
109. Kim DY, Mackenzie Ingano LA, Carey BW, Pettingell WP, Kovacs DM. Presenilin/gamma-secretase-mediated cleavage of the voltage-gated sodium channel beta 2 subunit regulates cell adhesion and migration. *J Biol Chem* 2005; 280:23251-61.
110. Diss JK, Stewart D, Fraser SP, Black JA, Dib-Hajj S, Waxman SG, et al. Expression of skeletal muscle-type voltage-gated Na<sup>+</sup> channel in rat and human prostate cancer cell lines. *FEBS Lett* 1998; 427:5-10.
111. Patino GA, Isom LL. Electrophysiology and beyond: Multiple roles of Na(+) channel beta subunits in development and disease. *Neurosci Lett* 2010; 486:53-9.
112. Malhotra JD, Kazen-Gillespie K, Hortsch M, Isom LL. Sodium channel  $\beta$  subunits mediate homophilic cell adhesion and recruit ankyrin to points of cell-cell contact. *J Biol Chem* 2000; 275:11383-8.



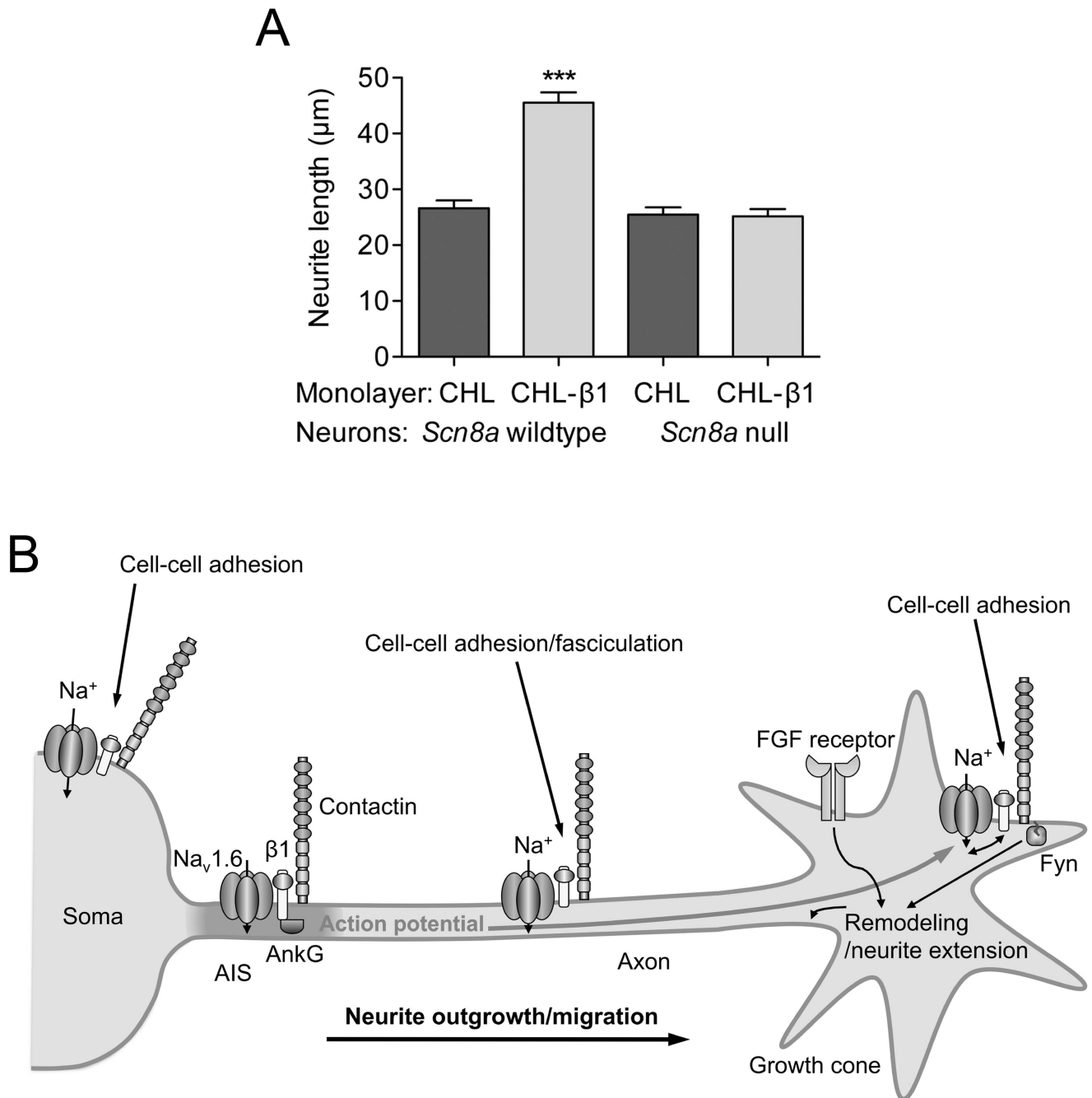
**Figure 1.** Multifunctional interactions of VGSCs. Basic topology of the pore-forming  $\alpha$  subunit is shown, consisting of four homologous domains each containing six transmembrane segments. Segment 4 contains the voltage sensor.<sup>6</sup> The smaller  $\beta$  subunits contain an extracellular immunoglobulin (Ig) loop, transmembrane domain, and an intracellular C-terminal domain.<sup>88</sup>  $\beta 1$  and  $\beta 3$  are non-covalently linked to the  $\alpha$  subunit, whereas  $\beta 2$  and  $\beta 4$  are covalently linked through disulfide bonds.<sup>2</sup> The alternative splice variant,  $\beta 1B$ , lacks a transmembrane domain.<sup>89</sup>  $\alpha$  subunits interact with a number of other signaling molecules, including p11,<sup>90</sup> protein kinase A (PKA),<sup>91</sup> protein kinase C (PKC),<sup>92</sup> ankyrin G,<sup>93</sup> MOG1,<sup>94</sup> fibroblast growth factor-homologous factor 1B (FHF1B),<sup>95</sup> calmodulin,<sup>96</sup> NEDD4,<sup>97, 98</sup> syntrophin,<sup>99</sup> and dystrophin.<sup>100</sup> Several of the  $\beta$  subunits interact with other cell adhesion molecules and regulatory proteins, including tenascin C,<sup>101</sup> tenascin R,<sup>101, 102</sup> contactin,<sup>103</sup> N-cadherin,<sup>104</sup> neurofascin (NF)155,<sup>105</sup> NF186,<sup>105</sup> NrCAM,<sup>105</sup> ankyrin G,<sup>104</sup> receptor protein tyrosine phosphatase  $\beta$  (RPTP $\beta$ ),<sup>106</sup> PKA,<sup>107</sup> and fyn kinase.<sup>68</sup> The  $\beta$  subunits are substrates for proteolytic cleavage by  $\alpha$ -secretase, BACE1, and  $\gamma$ -secretase.<sup>108, 109</sup> The intracellular domain of  $\beta 2$  is proposed to regulate gene expression in the nucleus.<sup>71</sup>  $\psi$ , glycosylation sites. Figure was produced using Science Slides 2006 software.



**Figure 2.**  $\alpha$  subunit involvement in pH-dependent cellular invasion.  $\text{Na}^+$  influx through  $\text{Na}_v1.5$  is proposed to activate the  $\text{Na}^+/\text{H}^+$  exchanger NHE1, co-expressed with  $\text{Na}_v1.5$  in lipid rafts contained within the caveolae of invasive breast cancer cells.<sup>59</sup> Increased NHE1 activity results in increased  $\text{H}^+$  efflux, which in turn enhances proteolytic activity of cysteine cathepsins B and S, which degrade the extracellular matrix, permitting invasion.<sup>52, 59</sup> The mechanism by which  $\text{Na}_v1.5$  enhances NHE1 is not yet clear. Figure was produced using Science Slides 2006 software.



**Figure 3.**  $\text{Na}_v1.5$ -regulated gene transcriptional network controlling invasion. Oval nodes represent regulatory genes, gray boxes represent effector genes, and white boxes represent gene ontology categories. Arrows indicate activation, and tees show repression. Reprinted by permission from the American Association for Cancer Research: House CD *et al*, Voltage-gated  $\text{Na}^+$  channel *SCN5A* is a key regulator of a gene transcriptional network that controls colon cancer invasion, *Cancer Research*, Sep 1, 2010, 70, 17, 6957-67, 10.1158/0008-5472.CAN-10-1169.



**Figure 4.** Neurite outgrowth and excitability regulated by  $\beta 1$  and  $\text{Na}_v1.6$ . (A)  $\beta 1$ -mediated neurite outgrowth is inhibited by the *Scn8a* null mutation. Neurite lengths of wild-type and *Scn8a* null cerebellar granule neurons grown on monolayers of control  $\beta 1$ -expressing fibroblasts. Data are mean and SEM. \*\*\* $P < 0.001$ . (B) Proposed model for  $\text{Na}^+$  current reciprocal involvement in  $\beta 1$ -mediated neurite outgrowth. Complexes containing  $\text{Na}_v1.6$ ,  $\beta 1$ , and contactin are present throughout the neuronal membrane in the soma, neurite and growth cone.  $\text{Na}^+$  influx is required for  $\beta 1$ -mediated neurite extension and migration. VGSC complexes along the neurite participate in cell-cell adhesion and fasciculation.  $\beta 1$  is also required for  $\text{Na}_v1.6$  expression at the axon initial segment, and subsequent high-frequency action potential firing. Electrical activity may further promote  $\beta 1$ -mediated neurite outgrowth at or near the growth cone. Figure reproduced with permission.<sup>56</sup>

**Table 1.** VGSC subtype expression in cancer.(A)  $\alpha$  subunits.

Protein	Gene	TTX sensitivity	Cancer type	References
Na <sub>v</sub> 1.1	<i>SCN1A</i>	Sensitive	Ovarian	17
Na <sub>v</sub> 1.2	<i>SCN2A</i>	Sensitive	Cervical, mesothelioma, ovarian, prostate	9, 14, 17, 18
Na <sub>v</sub> 1.3	<i>SCN3A</i>	Sensitive	Ovarian, prostate, small cell lung cancer	17, 18, 22
Na <sub>v</sub> 1.4	<i>SCN4A</i>	Sensitive	Cervical, ovarian, prostate	9, 17, 110
Na <sub>v</sub> 1.5	<i>SCN5A</i>	Resistant	Breast <sup>1</sup> , colon <sup>1</sup> , lymphoma <sup>1</sup> , neuroblastoma <sup>1</sup> , non-small cell lung cancer, ovarian, small cell lung cancer	8, 11, 15-17, 22, 26
Na <sub>v</sub> 1.6	<i>SCN8A</i>	Sensitive	Breast, cervical, lymphoma, melanoma, mesothelioma, non-small cell lung cancer, prostate, small cell lung cancer	8-10, 12, 14, 16, 18, 22, 26
Na <sub>v</sub> 1.7	<i>SCN9A</i>	Sensitive	Breast, cervical, lymphoma, mesothelioma, non-small cell lung cancer, ovarian, prostate <sup>1</sup>	8, 9, 14, 16-18, 26
Na <sub>v</sub> 1.8	<i>SCN10A</i>	Resistant	-	-
Na <sub>v</sub> 1.9	<i>SCN11A</i>	Resistant	Lymphoma, small-cell lung cancer	22, 26

(B)  $\beta$  subunits.

Protein	Gene	Cancer type	References
$\beta 1$	<i>SCN1B</i>	Breast <sup>1</sup> , cervical <sup>1</sup> , non-small cell lung cancer, prostate <sup>1</sup>	10, 16, 36, 38
$\beta 2$	<i>SCN2B</i>	Breast, cervical, non-small cell lung cancer, prostate	10, 16, 36-38
$\beta 3$	<i>SCN3B</i>	Non-small cell lung cancer, prostate	16, 36
$\beta 4$	<i>SCN4B</i>	Breast, cervical, non-small cell lung cancer, prostate	10, 16, 36, 38

<sup>1</sup>Cancers in which the indicated subunit has been reported as predominant.

**Table 2.** Metastatic cell behaviors regulated by VGSCs.

Cellular activity	Cancer	Subunit(s) implicated	Reference(s)
Process extension	Breast, prostate	Na <sub>v</sub> 1.7, $\beta$ 1	38, 39
Galvanotaxis	Breast, prostate	Na <sub>v</sub> 1.5, Na <sub>v</sub> 1.7	8, 40
Lateral motility	Breast, mesothelioma, prostate	Na <sub>v</sub> 1.5, Na <sub>v</sub> 1.7, $\beta$ 1, $\beta$ 2	14, 30, 37, 38, 41
Transwell migration	Breast, prostate	Na <sub>v</sub> 1.5, Na <sub>v</sub> 1.7	8, 17, 42-44
Endocytic membrane activity	Breast, prostate, small cell lung cancer	Na <sub>v</sub> 1.5, Na <sub>v</sub> 1.7	8, 22, 45
Vesicular patterning	Breast, prostate	Na <sub>v</sub> 1.7	46, 47
Adhesion	Breast, prostate	Na <sub>v</sub> 1.5, Na <sub>v</sub> 1.7, $\beta$ 1, $\beta$ 2	37, 38, 48
Gene expression	Breast, colon, prostate	Na <sub>v</sub> 1.5, Na <sub>v</sub> 1.7, $\beta$ 1	11, 38, 43, 49
Invasion	Breast, cervical, colon, lymphoma, melanoma, non- small cell lung cancer, prostate	Na <sub>v</sub> 1.5, Na <sub>v</sub> 1.6, Na <sub>v</sub> 1.7, $\beta$ 2	7, 8, 10-12, 16, 17, 19-21, 26, 37, 42



**Table 3.** Complementary functions of VGSC  $\alpha$  and  $\beta$  subunits in neurons and cancer cells.

Role	$\alpha$ subunit	$\beta$ subunit
In neurons	<p>Action potential initiation/conduction.<sup>3</sup></p> <p>Activity-dependent axon guidance, dendrite growth, synapse formation, and neurite outgrowth during development.<sup>5</sup></p>	<p>Modulate <math>\text{Na}^+</math> current, electrical activity.<sup>111</sup></p> <p>Enhance cell-cell adhesion.<sup>112</sup></p> <p>Enhance neurite outgrowth, migration, pathfinding, fasciculation during development.<sup>68</sup></p> <p>Reciprocal regulation of <math>\alpha</math> subunit expression.<sup>56</sup></p>
In cancer cells	<p>Highly expressed in strongly metastatic cells.<sup>50</sup></p> <p>Enhance metastatic behaviors including invasion, migration.<sup>5</sup></p> <p>Activity-dependent regulation of <math>\alpha</math> subunit expression by positive feedback.<sup>43, 78</sup></p>	<p>Highly expressed in weakly metastatic cells. Modulate <math>\text{Na}^+</math> current.<sup>37, 38</sup></p> <p>Enhance adhesion.<sup>37, 38</sup></p> <p>Enhance process extension.<sup>37, 38</sup></p> <p>Regulate migration.<sup>37, 38</sup></p> <p>Regulate <math>\alpha</math> subunit mRNA expression.<sup>38</sup></p>